

Some hints on the preparation and study of genitalia. (*With plate*).

By W. H. T. TAMS, F.E.S.

In reading from time to time papers, in which use is made of the study of genitalia, I have been struck by various remarks, which have led me to believe that it might be useful to some workers at least, if I published a short account of some of my own methods and observations.

We have still a long way to go, before we exhaust the interesting discoveries that await the investigator, in this most fascinating branch of entomological research. A glance at Rothschild and Jordan's *Revision of the Sphingidae* will convince any intending student of the value of an investigation of the genitalia in taxonomic work. There are numerous other works, which demonstrate the importance of the study of these structures, but it must not be taken for granted that they present an easy method of solving all the problems that confront the taxonomist. To rush blindly into the classification of a group purely on the morphology of the genital armature, because one investigator has succeeded in solving his difficulties in this way, will undoubtedly result in confusion. We have seen systems of classification in which, for instance, the rigid adherence throughout to the scheme laid down by the author concerned has led to the wide separation of closely allied species or genera, and it becomes increasingly evident that the only way to avoid falling into this trap is to test, as far as is possible, the value of the characters selected as a basis for the separation or grouping of species or genera. So we may see that the stalking of certain veins in the wings of insects may have an entirely different significance in different species or groups of species, and because two veins are separate in one species, the fact that they are stalked in another may not signify that the two species are not congeneric, though the same distinctions may have exactly the opposite significance in another group. The same observation is applicable in the case of the genitalia, and it may be pointed out in this connection, that it may be unwise to ignore the genitalia of one sex and to utilise characters based on modifications of the armature in the other sex alone for classificatory purposes.

Owing to the fact that many of the older authors were often misled by remarkable superficial resemblances to associate together species belonging to different genera, subfamilies, or even different families, it has become a common practice to regard these apparent indications of relationship as unimportant. It seems to me that it is much safer to place together two apparently closely related species, and then to exhaust all the means at one's disposal to test the value of the characters used in grouping, or separating, the species, in the particular subfamily concerned, than to select characters and to lay out a scheme of classification based on a knowledge of the value of such characters in other groups, regardless of the incongruous assemblage of species which may result. There are treatises in which the result of such a practice may be observed, and they will provide a happy hunting-ground for the inquirer, who would test the comparative values of characters, whether of neurulation, genitalia, scaling or superficial characters such as pattern, colour, etc. Colour may also prove most useful in associating the sexes of one species; but there are many cases, in which to trust to colour would lead to hopeless confusion.

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From the foregoing remarks it will be evident, then, that all available characters should be studied, and moreover, studied with a view to testing their comparative value. That the genital armature, both male and female, exhibits in many groups valuable characters that may be used both in separating and grouping species, has been abundantly demonstrated in various works, but it has been found that cases exist, in which these structures do not provide such characters.

The investigation of the genitalia in what is now regarded as a single species, may provide a valuable contribution to our knowledge of that and closely related species. Similar investigation of a whole genus would probably often provide even more valuable additions to our knowledge. Apparently unpretentious investigations of this nature may provide us with valuable aid, and may in some cases be as much as a student may find himself able to undertake. But the sooner our knowledge reaches the stage at which it covers much wider fields, the sooner shall we be able to assign proper values to the characters available to the taxonomist, and in this sphere of activity those who are able to undertake investigation on a small scale only, may be in the position to render most valuable and welcome assistance.

As some of the researchers already engaged in this type of investigation use methods differing from my own, an account of the procedure I adopt may lead others to take up work of this kind.

The first method I propose to describe is that used in preparing the male genitalia of *Sphingidae*. The procedure in this case does not entail the removal of the abdomen. Wood naphtha is applied to the terminal segments of the abdomen in order to render the membranes soft (a well-known method of relaxing somewhat amplified), and a fine needle-knife is then inserted between the organs and the walls of the eighth segment. With care the whole armature, comprising the ninth and tenth segments, to which the various parts of the genitalia are more or less firmly fixed, may be loosened sufficiently to make it possible to grip them with forceps and to withdraw them without injuring them. They may then be mounted dry (Dr. Jordan's method), or placed in potash and treated in the manner described below for balsam mounting. I have actually cleaned Sphingid genitalia in potash, afterwards washing out the potash in the ordinary way, dehydrating and then mounting them dry on celluloid, using cement made of celluloid dissolved in amyl acetate, a most useful preparation. Dr. Jordan uses a specially made card-board mount, consisting of two pieces of card, each with a small celluloid or glass window covering an oval hole punched through the card, the two pieces of card forming a capsule, and held together by the pin on which they are mounted. (fig. 1).

Another useful mount consists of a small celluloid capsule of two pieces, one fitting in the other. This can be fastened to a strip of celluloid with the celluloid and amyl acetate cement previously mentioned, and when the genitalia are enclosed the capsule can be lightly sealed with a touch of the cement. (fig. 2).

It is not so easy to remove the genitalia satisfactorily from the abdomen in most other families of moths as it is in the case of the *Sphingidae*, and other methods must be adopted. Whilst the abdomen, taken as a whole makes rather a large mount, there are some families in which it is advantageous to mount the whole abdomen, *e.g.*, in the

Pyralidae and *Psychidae*. The whole abdomen should always be examined before it is decided to discard any portion of it. Apart from the actual genital armature, consisting of the ninth and tenth segments and the various attached processes, particular attention should be paid to the eighth segment, which is often modified, especially the eighth ventrite. This I have found most important in the *Lasiocampidae*.

One of the reasons why I have been induced to write this short account, is the fact that I have several times heard it said, and at least once have seen it stated in print, that the membranous parts become fragile or are lost in the preparation of the specimen. The reason for this I believe to be that nearly every worker regards it as necessary to *boil* the specimen in potash (10%). I can state without fear of contradiction that perfectly satisfactory preparations can be made without the use of *hot* potash, and that with the more delicate insects it is often advantageous to dilute the 10% potash. By avoiding heat, and taking the specimen out of the potash as soon as it is soft enough to handle and dissect, the connecting membranes do not become inconveniently fragile and can be handled with forceps without the danger of tearing them.

My practice is as follows. If the specimen is unique, it is desirable to preserve the abdomen. In the case of hairy-bodied moths like the *Lasiocampidae* this is comparatively easy, but with moths having scale-covered bodies, as for example *Noctuidae* and *Geometridae*, this is more difficult and great care is needed in order to remove the genital armature without rubbing the scales from the abdomen. In those cases in which it is desired to preserve the abdomen, this is never put in potash. Maceration in water is the method used. As the abdomen will not readily absorb water at once, it should be dipped in alcohol (70% to 95%), and the alcohol will immediately permeate the entire specimen, which should then be drained by rolling it on blotting paper, after which it may be dropped into a tube containing water.

Twelve to thirty-six hours will suffice to soften most specimens (usually the shorter time is sufficient). The trace of alcohol prevents advanced maceration and allows softening only. The genital armature (with the eighth segment, or the eighth ventrite only, where necessary) may then be dissected out with ease and transferred to potash for the purpose of cleaning and clearing it of extraneous matter. It is important to note that an excess of alcohol apparently hinders the softening of the muscles, and particular attention should be paid to draining it off on blotting paper. The method of treating the specimen after it has been placed in potash only differs from that of treating specimens placed straight in potash, in that it is not necessary to leave them in that fluid for so long. With regard to the abdomen, as soon as the necessary portion has been extracted, the remainder is transferred at once to 95% alcohol, and then roughly dehydrated by being placed for a short time in absolute alcohol, after which it may be allowed to dry. For drying purposes a row of pins may be stuck through a cardboard box, points upwards, and the specimens placed on the points until they are dry, when they may be reattached to the moths to which they belong. (fig. 3).

When, owing to the availability of plenty of material, the saving of time and of absolute alcohol is of greater importance than the

preservation of a single abdomen, the abdomen, after being dipped in alcohol (and drained), to ensure rapid penetration, is placed directly into potash. In the case of large moths, *e.g.*, *Saturniidae*, *Lasiocampidae*, etc., twelve hours in potash is advisable. The genitalia of *Noctuidae* may be dissected out after two or three hours in 10% potash, but they make better preparations if, after they are removed from the abdomen, they are soaked in potash, or even in water, for a few hours longer. It is sometimes a distinct advantage to dissect them out and give them a further soaking, as it will sometimes be found that they are held in a solid concretion within the abdomen, almost as if they were set in plaster. Small *Noctuidae*, *Geometridae*, and most *Pyralidae* and such small moths are better treated with diluted potash, and may usually be ready to dissect or clean after three or four hours in a weak solution. The judgment of the manipulator may be exercised as to the advisability of preserving and mounting the whole abdomen.

After the specimen has soaked sufficiently in potash, it is most convenient to dissect out the genitalia, and to remove unwanted scales or hair-scales, in alcohol (70% to 95%), and not in water. The preparations are handled much more readily in alcohol, which in addition appears to remove the potash much more rapidly. Acid alcohol may be used with advantage. Twenty-four hours in clean 95% alcohol takes out most of the potash, and with two or three changes through clean spirit it may be completely removed. The preparation should then be dehydrated by soaking for twenty-four hours in absolute alcohol, after which it may be taken through xylol and mounted in balsam, or it may be kept in cedar-wood oil or clove oil. It is often a great advantage to keep preparations unmounted until they have been studied from every point of view, after which they may be mounted in the most suitable position for comparison with those of related species. Another method that may be used is to take the preparation straight from alcohol to glacial acetic acid, from which it may be mounted in balsam.

With regard to mounting in balsam, this may be done in the usual way with cold balsam, or with practice hot balsam may be used. Mounting in hot balsam has the great advantage that the preparation is held steady as soon as the balsam cools, and if it is found desirable to change its position, a hot needle inserted beneath the cover-glass, after the slide has been slightly warmed, will serve to effect the necessary rearrangement. A great disadvantage of hot balsam is that it becomes dark in colour through the effect of heat on the xylol in which it is dissolved, but it is possible that with some other solvent this might be avoided.

In the case of one or two families of moths the chitin of the genital armature is not only of a dark colour, but the scales and hair-scales are also dark in colour and often firmly attached to the chitin. Lengthy immersion in potash does not seem to materially reduce the intensity of colour, but if the preparations are taken straight from potash and placed in a small quantity of alcohol in a tube, prolonged immersion will steadily reduce the colour to the required degree of translucency. This method is particularly useful in the case of *Syntomidae*, some *Arctiidae*, and *Zygaenidae*.

It is often advisable to separate parts of the genitalia in order to get a better view of some particular structure, and this provides a

weighty argument against the hasty mounting of the whole preparation without a careful preliminary examination and comparison with the armatures of related forms. As a rule it is not difficult to remove one valve, and the aedeagus is usually easily separated. The vesica of the aedeagus is not always expanded, but with careful manipulation this can be effected without much difficulty, and without damage to the delicate membrane.

A hint worth remembering in photographing genitalia mounted in balsam, is that the surface of which it is desired to record the details, should be towards the plate, *e.g.*, if it is desired to photograph a valve (clasp) of the genitalia of an Agrotid (*Noctuidae*), the inner aspect, with the harpe, should face the plate. The same remarks apply in the case of making drawings by projection.

In conclusion, I should like to point out that a simple line drawing of a valve or even of a portion of a valve may often prove of greater value as an aid to identification than a page of letter-press and a coloured plate of the whole insect. One need only mention the case of *Acrionicta tridens*, S. & D., and *A. psi*, Linn. as an outstanding example.

Two species of Myrmecophilous Coleoptera New to Britain.

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(1). *Tachyusida gracilis*, Er.

Oxyopoda gracilis, Erichson, W. F. [*Die Käfer der Mark Brandenburg* 1 351 (1837)].

"*O. gracilis*: Elongata, rufo-testacea, abdomine ante apicem, elytris capiteque pices centibus: thorace basin versus angustato, canaliculato, margine reflexo.—Long. $1\frac{1}{2}$ lin."

There follows a very good description in German. Erichson was evidently not satisfied that this beetle belonged to *Oxyopoda*, as he says that from this genus it was very distinguishable, especially by the narrow form and unusually long legs and antennae. Fairmaire and Laboulbène placed it in the genus *Leptusa*. In 1872 Rey [*Ann. Soc. Lin. Lyon* 19 278 (1872)] erected the genus *Tachyusida* for this species. Reitter [*Fauna Germanica* 2 81 (1909)] points out that it differs from *Leptusa* by the strong lengthened basal joint of the antenna, the very long first joint of the hind tarsi, and from all other genera of this tribe (*Bolitocharini*—the tarsal formula is 4, 4, 5) by the heart-shaped thorax which is not bent under at the sides, with a distinct side border-line above. He says it has been taken in old tree trunks in the Rhein province, and East Germany, and is very rare. The European Catalogue of Coleoptera gives Germany, France, and Hungary.

This beetle is undoubtedly a myrmecophilous species, and more-over a guest of the ant *A. (D.) brunneus*, Latr. It is worthy of notice that in the European Catalogue of Coleoptera (1906) the genus *Tachyusida* (*T. gracilis*, Er., is the only species found in Europe, and single species occur in N. America, Japan, and India) is placed next to *Euryusa*, of which all the European species are guests of *brunneus*. I have little doubt that the old tree trunks recorded, were, or had been, inhabited by this ant.

On October 12th, an old dead oak tree in Windsor Forest, which I had known for several years to be inhabited by a strong colony of